

Acute changes in fibrinogen metabolism and coagulation after hemorrhage in pigs

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Submitted 25 March 2005; accepted in final form 10 June 2005

Martini, Wenjun Z., David L. Chinkes, Anthony E. Pusateri, John B. Holcomb, Yong-Ming Yu, Xiao-Jun Zhang, and Robert R. Wolfe. Acute changes in fibrinogen metabolism and coagulation after hemorrhage in pigs. *Am J Physiol Endocrinol Metab* 289: E930–E934, 2005. First published June 14, 2005; doi:10.1152/ajpendo.00137.2005.—Hemorrhagic coagulopathy is involved in the morbidity and mortality of trauma patients. Nonetheless, many aspects of the mechanisms underlying this disorder are poorly understood. We have therefore investigated changes in fibrinogen metabolism and coagulation function after a moderate hemorrhagic shock, using a new stable isotope approach. Twelve pigs were randomly divided into the control (C) and hemorrhage (H) groups. Hemorrhage was induced by bleeding 35% total blood volume over a 30-min period. A primed constant infusion of [1-¹³C]phenylalanine (Phe), d₅-phenylalanine, and α-[1-¹³C]-ketoisocaproate (KIC) was given to quantify fibrinogen synthesis and breakdown, together with measurements of circulating liver enzyme activities and coagulation function. Mean arterial pressure was decreased by hemorrhage from 89 ± 4 mmHg in C to 47 ± 4 mmHg in H (*P* < 0.05), followed by a rebound to 68 ± 5 mmHg afterward. Fibrinogen fractional synthesis rate increased from 2.7 ± 0.2%/h in C to 4.2 ± 0.4%/h in H by Phe (*P* < 0.05) and from 3.1 ± 0.4%/h in C to 4.4 ± 0.5%/h in H by KIC (*P* < 0.05). Fibrinogen fractional breakdown rate increased from 3.6 ± 1.0%/h in C to 12.9 ± 1.8%/h in H (*P* < 0.05). The absolute breakdown rate accelerated from 3.0 ± 0.4 mg·kg⁻¹·h⁻¹ in C to 5.4 ± 0.6 mg·kg⁻¹·h⁻¹ in H (*P* < 0.05), but the absolute synthesis rate remained unchanged. These metabolic changes were accompanied by a reduction in blood clotting time to 92.7 ± 1.6% of the baseline value by hemorrhage (*P* < 0.05). No changes were found in liver enzyme activities. We conclude that the observed changes in coagulation after hemorrhagic shock are mechanistically related to the acute acceleration of fibrinogen degradation.

stable isotopes; fibrinogen metabolism; hemorrhage; coagulation

HEMORRHAGIC COAGULOPATHY (without neurological injury) constitutes 40% of injury-related death in civilian hospitals and on the battlefield (1, 3, 15, 18). Hemorrhagic coagulopathy includes an initial hypercoagulable state that may result in consumptive depletion of fibrinogen and progress to disseminated intravascular coagulation (DIC; see Refs. 2, 7, 8, 11, 12, 18, 22). DIC results in a paradoxical situation in which patients are hypocoagulable in terms of hemostasis and therefore cannot stop bleeding, and yet inappropriately deposit fibrin in the microvasculature, contributing to later organ failure in patients that survive the initial hemorrhagic insults. It appears that the availability and metabolism of fibrinogen may play a role in the development of the pathophysiological process. However, the

relation between changes in fibrinogen metabolism to the development of clotting defects is poorly understood. The complexity of the clinical settings, such as tissue injury, blood loss, blood transfusion, and resuscitation, makes it difficult to clarify the mechanism contributing to the development of clotting disorders. Therefore, we have used an animal model that allowed us to define changes in fibrinogen metabolism under hemorrhage shock.

The essence of the coagulation process is the formation of fibrin from its precursor fibrinogen. Normal physiological coagulation requires a dynamic balance of complex systems of procoagulant, anticoagulant, and fibrinolytic processes. The pathophysiological states involve dysfunction of these processes. Investigation of coagulation in trauma patients has been primarily based on hematological tests from clinical laboratories that assess levels of procoagulant and anticoagulant factors (1, 7, 12, 22). Among all the factors involved in the coagulation process, fibrinogen level has been reported to be the first to drop to pathophysiological levels in patients with coagulation defects (1, 6). The drop cannot be fully explained by blood loss and resuscitation (6). It is not clear whether the drop in fibrinogen level is the result of inhibited production, accelerated consumption, or both, since there has not been an *in vivo* technique available for simultaneous quantification of both production and consumption.

This study was designed to develop an *in vivo* technique to quantify fibrinogen synthesis and degradation simultaneously and independently. Using this technique, we investigated changes in fibrinogen metabolism in parallel to the changes in the coagulation process after a moderate hemorrhagic shock in a swine model. Changes in fibrinogen synthesis were assessed using a 6-h infusion of α-[1-¹³C]ketoisocaproate ([1-¹³C]KIC) and [1-¹³C]phenylalanine ([1-¹³C]Phe), and changes in fibrinogen breakdown were assessed by a 4-h infusion of d₅-phenylalanine (d₅-Phe). We tested the hypothesis that fibrinogen breakdown was accelerated and fibrinogen synthesis remained unchanged after hemorrhage. To correlate fibrinogen metabolic changes with functional changes in coagulation, thromboelastography (TEG) was used to measure changes in clot formation and fibrin lysis from fresh whole blood samples.

METHODS

Experimental design. This study was approved by the US Army Institute of Surgical Research Institutional Animal Care and Use Committee. Twelve pigs were randomly allocated to normal control

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1. REPORT DATE 01 NOV 2005		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Acute changes in fibrinogen metabolism and coagulation after hemorrhage in pigs				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Martini W. Z., Chinkes D. L., Pusateri A. E., Holcomb J. B., Yu Y. M., Zhang X. J., Wolfe R. R.,				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX 78234				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 5	19a. NAME OF RESPONSIBLE PERSON
a REPORT unclassified	b ABSTRACT unclassified	c THIS PAGE unclassified			

(C; $n = 6$, 40.5 ± 1.6 kg) or hemorrhage (H; $n = 6$, 42.0 ± 0.8 kg) groups. After an overnight fast, animals were preanesthetized with glycopyrrolate (0.1 mg/kg) and Telazol (6 mg/kg), followed by 1.5–2.5% isoflurane in 100% oxygen by mask for the surgical procedures. A multiple sensor (Paratrend Diametrics Medical, High Wycombe, UK) was inserted in the carotid artery for measuring mean arterial pressure, temperature, pH, and heart rate. A Swan-Ganz catheter was inserted in the left jugular vein to measure cardiac output. The right femoral artery was cannulated for blood sampling and induction of bleeding. The left femoral vein was cannulated for stable isotope infusion, and the right femoral vein was cannulated for intravenous anesthesia of ketamine during the study.

Upon completion of surgical procedures, lactated Ringer solution was infused at $0.04 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ as a maintenance fluid in both groups throughout the study. Anesthesia was switched to a combination of isoflurane (0.5%) and continuous intravenous drip of ketamine ($0.15 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ of 100 mg/ml) throughout the entire study period. After 10-min stabilization, blood samples were taken from the femoral artery for baseline measurements. Hemorrhagic shock was then induced in the hemorrhage group by bleeding $\sim 35\%$ total blood volume from the left femoral artery to a preweighed canister on a balance over a 30-min period. The rate of bleeding was controlled by adjusting the clamp on the left femoral artery catheter to maintain mean arterial pressure >40 mmHg during the bleeding. After removal of the blood, the animal's condition was continuously monitored for 30 min before starting a primed constant infusion of stable isotope tracers. Pigs in the control group were given the same amount of maintenance fluid and anesthesia, but not bled. No resuscitation or heparin was used in this study.

Stable isotope infusion. The protocol of the primed constant infusion of stable isotopes is graphically depicted in Fig. 1. A sterile solution of stable isotope-labeled $[1\text{-}^{13}\text{C}]\text{KIC}$, $[1\text{-}^{13}\text{C}]\text{Phe}$, and $\text{d}_5\text{-Phe}$ was made in 0.45% saline and infused via the left femoral vein. On starting the infusion study, a priming dose of stable isotopes was given via the femoral vein containing $[1\text{-}^{13}\text{C}]\text{KIC}$ ($30 \mu\text{mol/kg}$), $[1\text{-}^{13}\text{C}]\text{Phe}$ ($18 \mu\text{mol/kg}$), and $\text{d}_5\text{-Phe}$ ($18 \mu\text{mol/kg}$). This was followed immediately by a constant infusion of tracer $[1\text{-}^{13}\text{C}]\text{KIC}$ ($0.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), $[1\text{-}^{13}\text{C}]\text{Phe}$ ($0.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), and $\text{d}_5\text{-Phe}$ ($0.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

$\text{kg}^{-1} \cdot \text{min}^{-1}$). The infusion of $[1\text{-}^{13}\text{C}]\text{KIC}$ and $[1\text{-}^{13}\text{C}]\text{Phe}$ was maintained for 6 h, and the $\text{d}_5\text{-Phe}$ infusion was maintained for 4 h (Fig. 1). The total tracer solution infused over 6 h was $125 \pm 3 \text{ ml}$ in C and $114 \pm 6 \text{ ml}$ in H. Blood samples (10 ml each) were collected hourly for 6 h with two additional samplings at 5.5 and 6.5 h. At 5 h, a bolus dose of sterile indocyanine green (ICG) dye solution (10 ml of 2.5 mg/ml) was given via the femoral vein, and additional blood samples (2 ml each) were collected upon injection and then at 5, 10, and 15 min for the assessment of plasma volume. Additional blood samples were drawn at baseline and 5 h (1 ml each) for blood clotting measurements.

Analytical methods. Hematocrit (Hct) and platelet counts were measured from citrated blood using an ABX Pentra 120 Hematology Analyzer (ABX Diagnostics, Irvine, CA). Blood chemistry was measured using a Dimension Clinical Chemistry System (Dade Behring, Newark, DE) that included plasma total protein content and the enzyme activities of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and γ -glutamyltranspeptidase. Plasma fibrinogen concentration was measured using the BCS Coagulation System (Dade Behring, Deerfield, IL).

Blood coagulation process was evaluated from 1 ml fresh whole blood using a TEG 5000 Hemostasis Analyzer (Haemoscope, Niles, IL) with pig thromboplastin. In TEG measurements, blood clotting time is the time required for the formation of initial detectable clot in measuring cups and LY_{60} is an estimate of fibrin clot lysis.

For assessment of plasma free amino acid enrichments, 0.5 ml acidified plasma was loaded on a cation exchange column (AG 50W-X8 resin, 200–400 mesh, H^+ form; Bio-Rad). Amino acids were separated after elution with ammoniac hydroxide. The extracts were dried under speed vacuum and derivatized by *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide at 100°C for 1 h. Plasma fibrinogen was isolated by adding 0.5 M CaCl_2 and thrombin to form a fibrin clot, following the procedure described by Stein et al. (17). The purity of fibrinogen by this procedure has been validated previously by affinity chromatography (9) and by PAGE (10). The clot was then washed and hydrolyzed in 6 N HCl at 110°C for 24 h and dried under speed vacuum. The released amino acids after hydrolysis were isolated, dried, and derivatized in the same manner as for plasma free amino acids. The enrichments of leucine and phenylalanine from the plasma free amino acid pool and in fibrinogen protein were determined by gas chromatography-mass spectrometry (model 5973; Hewlett-Packard) in the electron impact ionization mode. A selective ion-monitoring method was used at nominal mass-to-charge ratio of 302 ($m + 0$) and 303 ($m + 1$) for leucine, and 336 ($m + 0$), 337 ($m + 1$), 338 ($m + 2$), 339 ($m + 3$), 340 ($m + 4$), and 341 ($m + 5$) for phenylalanine.

Calculations. Plasma fibrinogen fractional synthesis rate (FSR) was calculated using the formula

$$\text{FSR} = [\text{EB}_{(t_2)} - \text{EB}_{(t_1)}] / (\text{EF} \times t) \quad (1)$$

where $\text{EB}_{(t)}$ is the enrichment of fibrinogen-bound amino acids, EF is the precursor enrichment, t_1 is the time point when $\text{d}_5\text{-Phe}$ infusion was stopped (5 h), t_2 is the time point when infusion of $[1\text{-}^{13}\text{C}]\text{Phe}$ and $[1\text{-}^{13}\text{C}]\text{KIC}$ was stopped (7 h), and t is time. In using tracer $[1\text{-}^{13}\text{C}]\text{Phe}$ to calculate FSR, $\text{EB}_{(t)}$ was fibrinogen-bound phenylalanine enrichment and EF was plasma free phenylalanine enrichment at the steady state. In using tracer $[1\text{-}^{13}\text{C}]\text{KIC}$ to calculate FSR, $\text{EB}_{(t)}$ was fibrinogen-bound leucine enrichment, and EF was the plasma free leucine enrichment at the steady state.

Plasma fibrinogen fractional breakdown rate (FBR) was determined by calculating the fractional rate of loss of labeled fibrinogen after the $\text{d}_5\text{-Phe}$ tracer was stopped. When the $\text{d}_5\text{-Phe}$ tracer was stopped, the plasma phenylalanine enrichment did not immediately go to zero because it took time for the labeled phenylalanine to be cleared from the blood, so labeled fibrinogen continued to be produced during this period. To account for this continued label incorporation, we determined the predicted fibrinogen-bound $\text{d}_5\text{-Phe}$ enrichment [as-

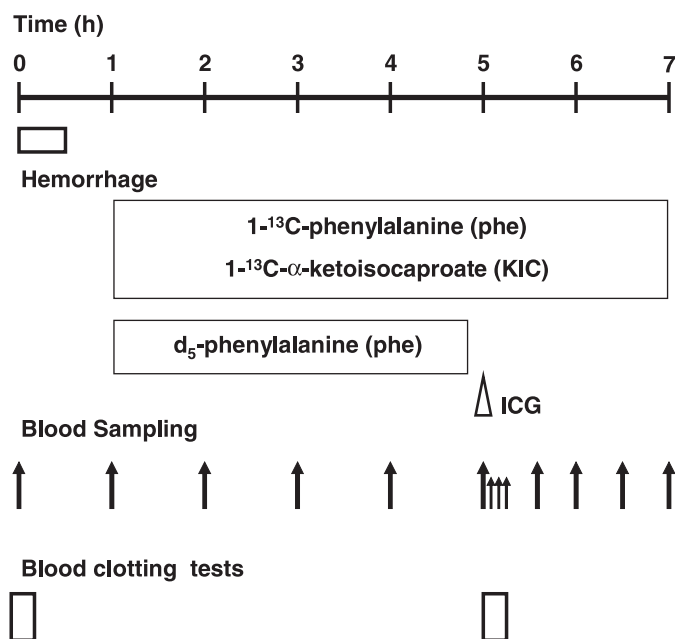


Fig. 1. Stable isotope infusion study protocol in control ($n = 6$ pigs) and hemorrhage ($n = 6$ pigs) groups. Phe, phenylalanine; KIC, ketosisocaproate; ICG, indocyanine green.

suming that there was no loss of labeled phenylalanine (denoted EB_{pred}) using the formula

$$EB_{pred} = EB_{(t1)} + FSR \times EF \times t \quad (2)$$

where $EB_{(t1)}$ is the starting fibrinogen bound phenylalanine enrichment when d_5 -Phe infusion was stopped, EF is the enrichment of plasma phenylalanine, and t is the amount of time that elapsed from the starting fibrinogen-bound phenylalanine measurement. To account for the delay between the time when plasma phenylalanine is taken up to the time that it appears in bound fibrinogen, EF is the free phenylalanine enrichment 1 h before the time of the fibrinogen-bound enrichment. Note that Eq. 2 is just a rearrangement of FSR Eq. 1 where EB_{pred} is $EB_{(t2)}$.

The actual measured fibrinogen-bound phenylalanine enrichment (denoted EB_{act}) will be lower than the above predicted enrichment to the extent that the label is irreversibly lost. Therefore, the FBR can be calculated using the formula

$$FBR = (EB_{pred} - EB_{act}) / [EB_{(t1)} \times t] \quad (3)$$

The numerator of this equation represents the amount of tracer that is irreversibly lost (relative to the amount of tracee). Dividing this value by the initial enrichment [$EB_{(t1)}$] gives the percentage of the initial enrichment that was irreversibly lost, and dividing also by the time gives the fractional rate that the tracer is lost. It is assumed that the fractional rate of loss of unlabeled fibrinogen is the same as the labeled fibrinogen.

The plasma fibrinogen absolute synthesis rate was calculated by multiplying FSR by plasma fibrinogen concentration and plasma pool size. Similarly, plasma fibrinogen absolute breakdown rate was calculated by multiplying FBR by plasma fibrinogen concentration and plasma pool size.

Statistical analysis. All results are expressed as means \pm SE. Comparisons between the groups in fibrinogen synthesis, breakdown, liver enzyme activities, substrate concentrations, and changes in blood clotting measurements were made with Student's *t*-test. Statistical significance was set at the 0.05 level.

RESULTS

Physiological data. All measurements in the control group remained unchanged during the study. Mean arterial pressure decreased immediately after hemorrhage from 89 ± 4 mmHg in C to 47 ± 4 mmHg in H ($P < 0.05$), followed by a rebound to 68 ± 5 mmHg in H after 30 min. Similarly, cardiac output decreased immediately after hemorrhage from 3.4 ± 0.4 l/min in C to 1.8 ± 0.2 l/min in H ($P < 0.05$), followed by a rebound to 2.9 ± 0.4 l/min in H after 30 min. Within 1 h after hemorrhage, there was a significant increase in blood lactate concentration and decreases in Hct, fibrinogen concentration, and plasma total protein content. Afterward, there were no changes within each group from 1 to 7 h in measurements of Hct ($29.2 \pm 0.7\%$ in C and $25.4 \pm 1.1\%$ in H, $P < 0.05$), platelet count ($316.7 \pm 33.7 \times 10^3/\text{ml}$ in C and $268 \pm 11.1 \times 10^3/\text{ml}$ in H, $P = 0.15$), fibrinogen concentration (185.8 ± 15.5 mg/dl in C and 120.6 ± 2.4 mg/dl in H, $P < 0.05$), and total protein concentration (5.14 ± 0.21 g/l in C and 3.91 ± 0.15 g/l in H, $P < 0.05$). Plasma volume, measured by ICG dye at 5 h, was 49.7 ± 0.5 ml/kg in C and 34.0 ± 0.4 ml/kg in H ($P < 0.05$).

Blood biochemical and clotting data. There were no differences between the two groups in circulating enzyme activities of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and γ -glutamyltranspeptidase during the study.

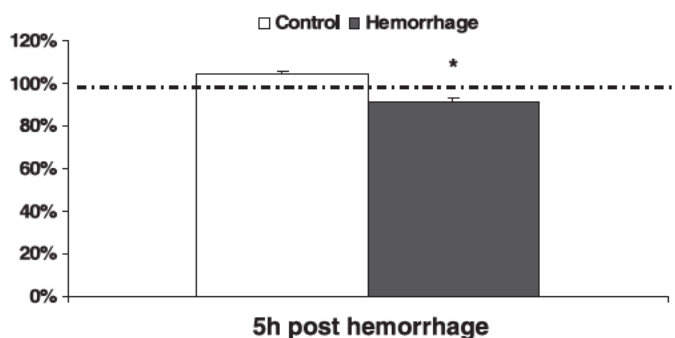


Fig. 2. Changes in blood clotting time as %baseline values in control ($n = 6$) and hemorrhage ($n = 6$) groups 5 h posthemorrhage shock. A decrease in clotting time indicates an acceleration of the clotting process. $*P < 0.05$ control vs. hemorrhage groups. Data are expressed as means \pm SE.

Changes of blood clotting time after hemorrhage are shown in Fig. 2. At 5 h, blood clotting time in H was shortened to $92.7 \pm 1.6\%$ of its baseline value compared with $104 \pm 1.5\%$ of its baseline value in C ($P < 0.05$). There were no differences in fibrin clot lysis (LY_{60}) between the two groups.

Isotope data and fibrinogen kinetics. The enrichments of plasma free leucine tracer-to-tracee ratio (TTR) ($m + 1$) in both groups reached plateau values ($9.37 \pm 0.35\%$ in C and $9.07 \pm 0.61\%$ in H) after 1 h of [$1\text{-}^{13}\text{C}$]KIC infusion (Fig. 3). Similarly, the enrichments of plasma free Phe TTR ($m + 1$) in both groups reached plateau values ($21.23 \pm 0.58\%$ in C and $23.80 \pm 1.30\%$ in H) after 1 h of [$1\text{-}^{13}\text{C}$]Phe infusion (Fig. 3). The calculated rate of appearance for phenylalanine, which is a reflection of whole body protein breakdown (21), was $1.42 \pm 0.04 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in C and $1.27 \pm 0.08 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in H ($P = 0.16$).

The enrichments of fibrinogen-bound phenylalanine TTR ($m + 1$) and leucine TTR ($m + 1$) showed a linear increase during the infusion of [$1\text{-}^{13}\text{C}$]Phe and [$1\text{-}^{13}\text{C}$]KIC (Fig. 4). Plasma fibrinogen FSR was calculated from the increment of fibrinogen enrichments between 5 and 7 h of the infusion study. Fibrinogen FSR measured by [$1\text{-}^{13}\text{C}$]KIC was $3.1 \pm 0.4\%/h$ in C and $4.4 \pm 0.5\%/h$ in H ($P < 0.05$), and the FSR by [$1\text{-}^{13}\text{C}$]Phe was $2.7 \pm 0.2\%/h$ in C and $4.2 \pm 0.4\%/h$ in H ($P < 0.05$). It is worth mentioning that, within each group, the FSR values measured by these two tracers were close, and significant increases of FSR by hemorrhage were shown by both tracers. Fibrinogen absolute synthesis rate, calculated by multiplying FSR by fibrinogen concentration and plasma volume, was $2.5 \pm 0.6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in C and $1.7 \pm 0.2 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in H by tracer [$1\text{-}^{13}\text{C}$]Phe ($P = 0.24$, Fig. 5) and $3.0 \pm 0.8 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in C and $1.8 \pm 0.2 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in H by tracer [$1\text{-}^{13}\text{C}$]KIC ($P = 0.43$).

Fibrinogen FBR was calculated from the changes in fibrinogen-bound Phe enrichment TTR ($m + 5$) after the cessation of tracer d_5 -Phe infusion (Fig. 4). The FBR in H was $12.9 \pm 1.8\%/h$, which was significantly accelerated from $3.6 \pm 1.0\%/h$ in C ($P < 0.05$). The absolute breakdown rate, calculated by multiplying FBR by fibrinogen concentration and plasma volume, was also accelerated to $5.4 \pm 0.6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in H compared with $3.0 \pm 0.4 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in C ($P < 0.05$, Fig. 5).

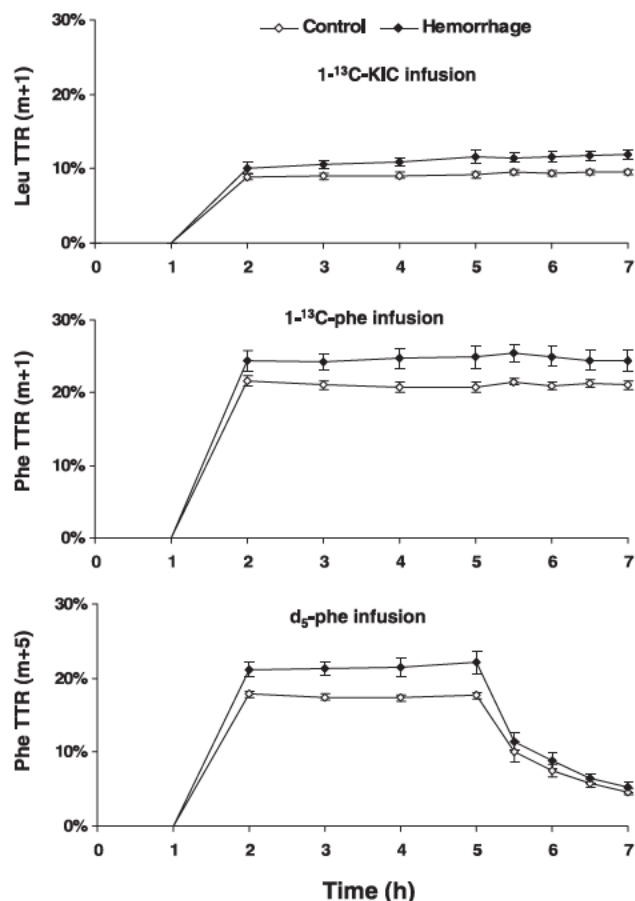


Fig. 3. Changes in plasma free leucine (Leu) and Phe enrichments in control (○, $n = 6$) and hemorrhage (●, $n = 6$) groups during the infusion of $\alpha\text{-}[1\text{-}^{13}\text{C}]\text{KIC}$ (6 h), $[1\text{-}^{13}\text{C}]\text{phenylalanine}$ ($[1\text{-}^{13}\text{C}]\text{Phe}$, 6 h), and $\text{d}_5\text{-phenylalanine}$ ($\text{d}_5\text{-Phe}$, 4 h). Data are expressed as means \pm SE. TTR, tracer-to-trace ratio.

DISCUSSION

We have developed a new stable isotope technique that allows, for the first time, simultaneous and independent quantification of fibrinogen synthesis and breakdown *in vivo*. The simultaneous quantification of synthesis and breakdown provides comprehensive understanding of changes in fibrinogen metabolism under different physiological conditions. Using this new technique, we investigated changes in fibrinogen metabolism in pigs after a hemorrhagic shock. Our finding was that, acutely after a moderate hemorrhagic shock, fibrinogen breakdown was accelerated, but synthesis remained unchanged. The acceleration in fibrinogen breakdown was associated with a shortening in blood clotting time. The deficit between fibrinogen production and consumption indicates a potential decrease in fibrinogen availability after hemorrhage.

Previous investigations of fibrinogen catabolism included ^{125}I -labeled fibrinogen administration and subsequent daily blood samplings for 5–14 days (4, 5, 16, 19, 20). The radioactivity decay curve during the sampling period was used to calculate fibrinogen turnover. This approach was used as the only *in vivo* means in the past to monitor clotting defects in patients and animals under pathophysiological states (4, 5, 16, 19, 20). As a comparison, there are a number of advantages of our new technique over the ^{125}I -fibrinogen method. First, we

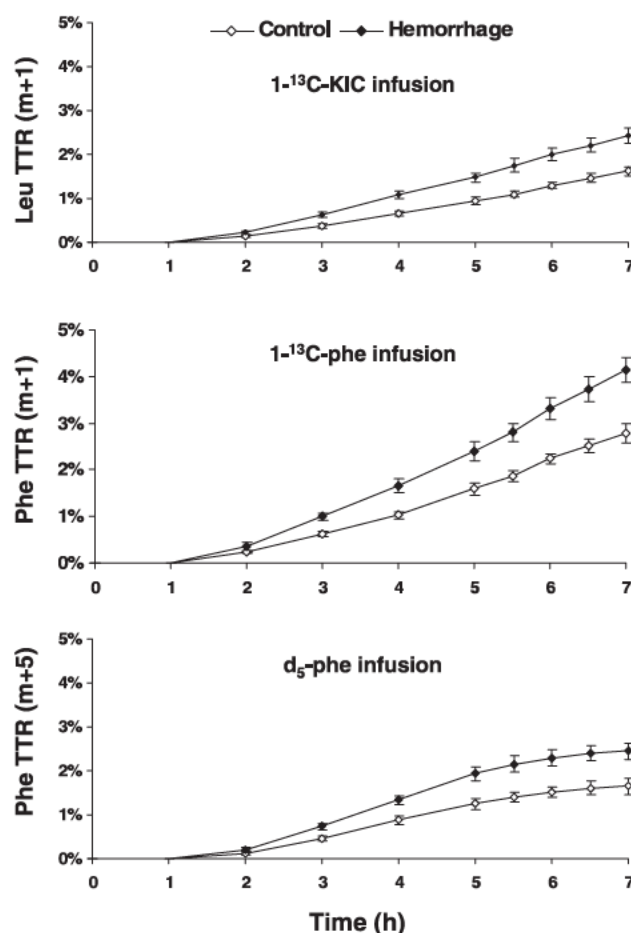


Fig. 4. Changes in fibrinogen-bound Leu and Phe enrichments in control (○, $n = 6$) and hemorrhage (●, $n = 6$) groups during the infusion of $[1\text{-}^{13}\text{C}]\text{KIC}$ (6 h), $[1\text{-}^{13}\text{C}]\text{Phe}$ (6 h), and $\text{d}_5\text{-Phe}$ (4 h). Data are expressed as means \pm SE.

used stable isotopes rather than radioactive isotopes, which is a safer approach. Second, we measure FSR and FBR independently and simultaneously, whereas studies using ^{125}I -fibrinogen only measure degradation. This is an important advantage in cases such as the present study in which synthesis and breakdown are not equal after hemorrhage. Third, our method provides fast and timely measurements to monitor the coagulation process, since it only takes 6 h as opposed to 5–14 days in the ^{125}I -fibrinogen approach. Fourth, unlike the ^{125}I -fibrinogen method, in which the exogenously given fibrinogen is not directly comparable to the endogenous fibrinogen, the labeled fibrinogen in this study is produced endogenously by incorporating naturally occurring stable isotopes. Furthermore, in the

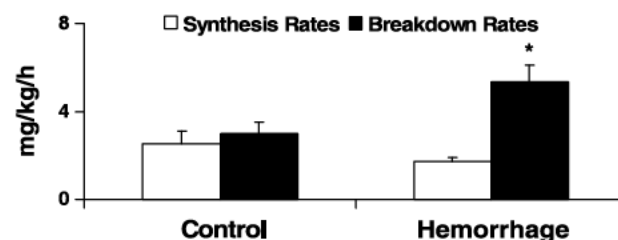


Fig. 5. Fibrinogen synthesis and breakdown rates in control ($n = 6$) and hemorrhage ($n = 6$) groups. * $P < 0.05$, control vs. hemorrhage groups. Data are expressed as means \pm SE.

previous ^{125}I -fibrinogen method, problems such as uneven labeling of exogenous fibrinogen, modification of the half-life of fibrinogen from the in vitro procedure, possible tag shifts between different proteins, and dilution from endogenous fibrinogen synthesis remain unresolved (13–14). All of these unresolved problems are likely to compromise the validity of fibrinogen degradation quantification using the ^{125}I -fibrinogen method.

Under the physiological situations in this study, the steady-state enrichment level of the infused tracer (Fig. 3) is mainly determined by the turnover rate of the tracee pool rather than the absolute concentration of the tracee in the metabolic pool (21); and the determination of FSR of fibrinogen is based on the slope of fibrinogen enrichment increment (Fig. 4) secondary to the steady-state plasma enrichment of the infused tracer (Fig. 3). It is also worth noting that none of the equations we used to calculate fibrinogen fractional synthesis or breakdown has a parameter of concentration. Therefore, we consider that, even if bleeding and dilution from the isotope infusion might alter precursor and product concentration to some degree, since the measurements were made under a quasi-steady state, our results of FSR and FBR should reasonably reflect the dynamic nature of fibrinogen metabolism. The absolute synthesis (or breakdown) rate was calculated as FSR multiplying plasma volume and fibrinogen concentration. Because plasma volumes and fibrinogen concentrations were directly measured in each animal group in this study, it is, therefore, not necessary to correct or adjust plasma volume for the assessments of absolute synthesis and breakdown rates.

In this study, $[1\text{-}^{13}\text{C}]\text{Phe}$, $\text{d}_5\text{-Phe}$, and $[1\text{-}^{13}\text{C}]\text{KIC}$ were infused at the same rates ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) in the control and hemorrhage groups. The total volume infused over 6 h was similar in control (125 ± 3 ml) and hemorrhage (114 ± 6 ml) groups. The similar volume of tracer might cause relatively more dilution in the hemorrhage group because of its blood loss before the isotope infusion. This additional dilution might possibly decrease clotting factor levels in hemorrhaged animals, resulting in possible prolonged clotting time compared with that in control animals. Thus our clotting time measurement, which was shortened $\sim 10\%$ after hemorrhagic shock, might possibly underestimate the hemorrhage effect on clotting time. However, because the infused volume during the 6 h was small compared with total blood volume, this possible underestimation was likely to be minimal. Therefore, we consider that our clotting time results are reasonable.

In summary, we developed a stable isotope model to quantify fibrinogen synthesis and breakdown independently and simultaneously in pigs. We found that, acutely after a moderate hemorrhage, fibrinogen breakdown was accelerated, whereas fibrinogen synthesis remained unchanged, resulting in a net loss of fibrinogen availability. This study provides an in vivo tool to investigate long-term or multifactorial effects on fibrinogen metabolism after hemorrhagic shock.

ACKNOWLEDGMENTS

We thank Suzanne Christensen, John Uscilowicz, Michael Scherer, and Douglas Cortez for technical assistance in animal studies and Amy Newland for assistance in editing the manuscript. We appreciate support received from

the Veterinary Service Support Branch and Laboratory Support Branch at the U.S. Army Institute of Surgical Research.

GRANTS

This study was supported by the U.S. Army Medical Research and Medical Command. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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